Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Claims 22 and 23 have been amended, claims 121–124 have been added, and claims 38–120 have been canceled. Support for the amendments and new claims is found in the present application at, *e.g.*, paragraphs [0038], [0047], and [0061], and Example 2. No new matter has been added. Claims 1–37 and 121–124 are pending.

The rejection of claims 22 and 23 under 35 U.S.C. § 112 for indefiniteness is respectfully traversed in view of the above amendments. In particular, claims 22 and 23 clearly recite that the method further comprises adding certain cells to the culture medium covering or surrounding the three-dimensional scaffolding.

The rejection of claims 1–30 under 35 U.S.C. § 102(a) as being anticipated by WO 01/036589 to Wu et al. ("Wu") is respectfully traversed.

As amended, claims 1–30 of the present application relate to a method of culturing peripheral lymphoid organ cells. This method involves isolating peripheral lymphoid organ cells from a peripheral lymphoid organ, where the peripheral lymphoid organ cells comprise lymphocytes; and culturing the peripheral lymphoid organ cells on a three-dimensional scaffolding which is covered or surrounded with culture medium under conditions effective to generate and maintain mature and functional peripheral lymphoid organ cells. The three-dimensional scaffolding allows cells in the culture medium to have cell to cell contact in three dimensions.

Wu teaches a cell culture system that includes a three-dimensional support for the culture of hematopoietic stem cells and stromal cells, and media that supports the growth or differentiation of the stem cells into immune system cells. Wu specifically describes culturing the mononuclear cell layer of human bone marrow in a three-dimensional bioreactor, and reports that the culture resembled the function of bone marrow *in vivo*. *See* Wu at 32–40.

The PTO's position is that Wu teaches a method of culturing peripheral lymphoid organ cells (that include lymphocytes) under conditions effective to generate and maintain mature and functional peripheral lymphoid organ cells. Applicants respectfully disagree.

The PTO has cited pages 4, 5, 9, and 18 of Wu as teaching the culture of peripheral lymphoid organ cells that include lymphocytes. They do not. Rather, Wu teaches methods for *producing* various immune system cells (*e.g.*, T lymphocytes, B lymphocytes).

These methods all involve culturing *hemopoietic stem cells* on a three dimensional support and allowing for the growth of, or *differentiation into*, immune system cells. The method described on page 4, lines 18–25, of Wu is representative:

> The present invention provides a method of producing immune system cells which comprises culturing stromal and hemopoietic stem cells on a three dimensional support and allowing for the growth of, or differentiation into, immune system cells.
>
> Examples of immune system cells produced by the methods of the present invention include, T lymphocytes, B lymphocytes, antigen presenting cells, natural killer cells, naive cells, activated cells, memory cells, and progenitors or precursors thereof.

The PTO correctly points out on page 3 of the office action that the cultures of Wu may contain lymphatic cells (*e.g.*, T and B lymphocytes) and that peripheral lymphoid organ cells include these cell types. However, the PTO is incorrect in concluding that Wu therefore teaches culturing peripheral lymphoid organ cells, because lymphatic cells are not necessarily peripheral lymphoid organ cells. Nowhere does Wu state that the lymphatic cells referred to therein are from peripheral lymphoid organs. On the contrary, as evidenced by the above-cited passage of Wu, the lymphatic cells of Wu are differentiated from cultured hemopoietic stem cells. The hemopoietic stem cells of Wu include "bone marrow stem cells, peripheral stem cells, embryonic stem cells, umbilical blood stem cells and other types of stem cells" (Wu, at p.14, ll.19–21), not peripheral lymphoid organ cells. Therefore, Wu does not teach a method of culturing peripheral lymphoid organ cells that include lymphocytes.

Also, the Second Declaration of Andrea Bottaro Under 37 C.F.R. § 1.132 accompanying this amendment demonstrates that the peripheral lymphoid organ lymphatic cells of this invention are functionally and phenotypically different from the lymphatic cells of Wu. The intrinsic differences between the lymphoid and accessory cell populations in bone marrow and lymph nodes are crucial to their respective functions, namely lymphopoiesis and the generation of antigen-specific adaptive immune responses. 2d Bottaro Decl., at ¶ 5. To highlight these differences, and test their effects on the outcome of immunization experiments in bioreactor culture systems, flow cytometry analysis was performed on fresh and cultured tonsil and bone marrow samples, and their responses to immunization to a common vaccine antigen, tetanus toxoid, were tested. *Id.* The bone marrow samples were cultured in a bioreactor as

described in Wu, while the tonsil samples were cultured in a bioreactor as described in this application. *Id.*

Flow cytometry was performed on fresh (*i.e.*, uncultured) bone marrow and tonsil samples to evaluate their cell population profiles. 2d Bottaro Decl., at ¶ 6. Figure 1 of Exhibit 1 of the Second Bottaro Declaration shows a comparison of these flow cytometry profiles. *Id.* Both tissues display substantial proportions of B and T lymphoid populations, although the abundance of B lymphocytes is lower in the bone marrow samples, to the expense of both T cells and, more notably, non-lymphoid populations (myeloid cells as well as erythroid precursors). *Id.* When analyzed for expression of IgD, a marker of naive, mature B cells, these constitute the largest population in the tonsil, but only a small fraction in the bone marrow. *Id.* (It is noted that in the bone marrow $CD19^+$, IgD^+ mature B cells represent a rapidly transient population of recirculating peripheral cells that are introduced via the blood circulation, but do not home to the bone marrow or reside there *in situ*. *Id.*)

To better characterize the B cell subsets in each organ, the samples were stained for the markers CD27 and CD38, which were analyzed after gating selectively on $CD19^+$ (B lymphoid) cells. 2d Bottaro Decl., at ¶ 7. This analysis revealed four cell populations: mature B cells, memory B cells, activated B cells in the tonsil, and, in the bone marrow, B cell precursors and plasma cells. *Id.* The latter population is predominantly detected in the bone marrow, the homing site for long-lived plasma cells generated in the periphery. *Id.*

Figure 2 of Exhibit 1 of the Second Bottaro Declaration shows the same type of analysis performed on tonsil and bone marrow samples cultured for two weeks in the bioreactor system. 2d Bottaro Decl., at ¶ 8. The most notable change compared to the fresh samples is the almost complete loss of the mature, naive B cell population in the bone marrow culture. *Id.* It is believed that this is because the bone marrow does not provide a suitable microenvironment for the recirculating mature B cells to survive in culture. *Id.* In contrast, IgD^+ cells are readily maintained in the tonsil cultures (experiments show that these cells can survive for 5–7 weeks in the system). *Id.* The residual B lymphoid population in the bone marrow bioreactors is comprised predominantly of the long-lived plasma cells highlighted in the fresh sample, while the other subsets (mostly precursor B cells) are comparatively under-represented. *Id.*

The loss of the mature naive population, the cell pool from which the humoral immune response precursors are selected after antigen stimulation, has significant implications

for the functional potential of bone marrow cultures. 2d Bottaro Decl., at ¶ 9. To test this, 2-week-old bone marrow and tonsil cultures were immunized with tetanus toxoid ("TTC") antigen and lipopolysaccharide as a broad-spectrum adjuvant. *Id.* Two weeks after immunization, following an antigen boost, the number of cells secreting IgM and IgG antibodies specific to TTC were measured using the Elispot technique. *Id.* As shown in Figure 3 of Exhibit 1 of the Second Bottaro Declaration, in tonsil samples, a 2- to >5-fold increase in the number of antigen-specific antibody-secreting cells of both classes is observed in successfully immunized cultures, while essentially no antigen-specific antibody secretors are detected in the immunized bone marrow sample. *Id.*

These results highlight the unique properties of peripheral lymphoid organ cultures with respect to both cell composition and the ability to respond to antigen stimulation. 2d Bottaro Decl., at ¶ 10.

Finally, nowhere does Wu teach or suggest isolating cells from a peripheral lymphoid organ, where the peripheral lymphoid organ cells include lymphocytes, as required by the present claims.

For all of these reasons, the rejection of claims 1–30 for anticipation by Wu is improper and should be withdrawn.

The rejection of claims 1–7 and 13–23 under 35 U.S.C. § 102(b) as being anticipated by WO 99/15629 to Pykett et al. ("Pykett") or U.S. Patent No. 5,160,490 to Naughton et al. ("Naughton") is respectfully traversed.

Pykett relates to a method for the long term *in vitro* culture of hematopoietic progenitor cells to produce differentiated cells of hematopoietic origin by culturing the cells on a three-dimensional porous material. Cells that can reportedly be produced according to this method include T cells, plasma cells, erythrocytes, megakaryocytes, basophils, polymorphonuclear leukocytes, monocytes, macrophages, eosinophils, and platelets.

Naughton relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues *in vitro* for prolonged periods of time. Tissues that can reportedly be cultured in this system include bone marrow, skin, liver, pancreas, kidney, adrenal, and neurological tissue. Cells that can reportedly be cultured include endothelial cells, macrophages, monocytes, B and T lymphocytes, and stromal cells.

Like Wu, Pykett and Naughton do not teach isolating cells from a peripheral lymphoid organ, where the peripheral lymphoid organ cells include lymphocytes, as required by the present claims. Accordingly, for substantially the reasons noted above, the rejection of claims 1–7 and 13–23 for anticipation by Pykett or Naughton is improper and should be withdrawn.

The rejection of claims 1, 6, 8–12, and 24–30 under 35 U.S.C. § 103(a) for obviousness over Pykett or Naughton in view of U.S. Patent No. 6,821,778 to Engelman et al. is respectfully traversed. Engelman has been cited for teaching a culture medium that contains antigens presented by antigen-presenting cells, but adding antigen-presenting cells to the culture media of Pykett and Naughton would not overcome their above-noted deficiencies. Accordingly, the rejection of claims 1, 6, 8–12, and 24–30 for obviousness over Pykett or Naughton in view of Engelman is improper and should be withdrawn.

In addition, in the office mailed August 1, 2006, claims 1–30 were provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 1–120 of U.S. Patent Publication No. 2005/0191743 to Wu et al. ("the '743 publication") and claims 1–106 of U.S. Patent Publication No. 2003/0109042 to Wu et al. ("the '042 application"). In the response filed February 1, 2007, it was noted this rejection was improper, because it was noted based on the claims of a co-pending application (*i.e.*, the '743 publication is the published form of the present application, while the '042 application has been abandoned). The PTO has noted on page 2 of the present office action that this rejection *vis-à-vis* the '042 application has been obviated, but no mention has been made of this rejection *vis-à-vis* the '743 publication. While it is assumed that the provisional double-patenting rejection of claims 1–30 has been withdrawn in its entirety, clarification is respectfully requested.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Dated: May 16, 2008

/Shelley A. Jones/
Shelley A. Jones
Registration No. 53,081

Nixon Peabody LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603-1051
Telephone: (585) 263-1461
Facsimile: (585) 263-1600